Journal of Photochemistry & Photobiology, B: Biology 185 (2018) 215–222

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Photodynamic activity of Temoporfin nanoparticles induces a shift to the M1-like phenotype in M2-polarized macrophages

Zhenxin Zhu[a], Claudia Scalfi-Happ[a], Anastasia Ryabova[b,c], Susanna Gräfe[d,1], Arno Wiehe[d], Ralf-Uwe Peter[e], Victor Loschenov[b,c,f], Rudolf Steiner[a,f], Rainer Wittig[a,*]

[a] Institute for Laser Technologies in Medicine & Metrology (ILM) at Ulm University, Helmholtzstrasse 12, 89081 Ulm, Germany
[b] A.M. Prokhorov General Physics Institute, RAS, Vavilov str.38, 119991 Moscow, Russia
[c] Biospec Ltd, Krimskiy val. 8, 119049 Moscow, Russia
[d] biolitec research GmbH, Otto-Schott-Strasse 15, 07745 Jena, Germany
[e] Gefaess- und Hautzentrum Blaustein, Erhard-Groezinger-Strasse 102, 89134 Blaustein, Germany
[f] National Research Nuclear University, MEPhI Moscow Engineering Physics Institute, Moscow, Russia

ARTICLE INFO

Keywords:
Photodynamic therapy
Temoporfin
Foslip
Crystalline nanoparticle
Tumor-associated macrophage
Macrophage polarization

ABSTRACT

The monocyte/macrophage cell lineage reveals an enormous plasticity, which is required for tissue homeostasis, but is also undermined in various disease states, leading to a functional involvement of macrophages in major human diseases such as atherosclerosis and cancer. We recently generated in vivo evidence that crystalline, nonfluorescent nanoparticles of the hydrophobic porphyrin-related photosensitizer Aluminum phthalocyanine are selectively dissolved and thus may be used for specific fluorescent labelling of rejected, but not of accepted xenotransplants. This led us to hypothesize that nanoparticles made of planar photosensitizers such as porphyrins and chlorins were preferentially taken up and dissolved by macrophages, which was verified by in vitro studies. Here, using an in vitro system for macrophage differentiation/polarization of the human monocyte THP-1 cell line, we demonstrate differential uptake/dissolution of Temoporfin-derived nanoparticles in polarized macrophages, which resulted in differential photosensitivity. More importantly, low dose photodynamic sensitization using Temoporfin nanoparticles can be used to trigger M1 re-polarization of THP-1 cells previously polarized to the M2 state. Thus, sublethal photodynamic treatment using Temoporfin nanoparticles might be applied to induce a phenotypic shift of tumor-associated macrophages for the correction of an immunosuppressive microenvironment in the treatment of cancer, which may synergize with immune checkpoint inhibition.

1. Introduction

Solid tumors are complex agglomerates of cells which contain both transformed cancer cells as well as non-transformed cells. They evolve over time and may be seen as organ-like entities where different cell types interact under guidance of the cancer cells, aiming at the suppression of perturbations initiated by the host tissue, the immune system and therapeutic drugs [1]. The microenvironment within a tumor is a result of the complex interplay of the different cell types and is frequently dominated by an immunosuppressive milieu, which prevents the development of an efficient immune response [2]. Tumor-associated macrophages (TAM) play a key role in the conditioning of the tumor microenvironment because they react on external conditions such as reduced blood flow or hypoxia by phenotypic changes, resulting in differential chemokine profiles altering e.g. vascularization and the immune response [3–5]. TAM are predominantly polarized to an anti-inflammatory M2 phenotype, and the M1/M2 ratio of TAM was found to be associated with survival for different cancer types [6]. Consequently, re-polarization of TAM to the inflammatory phenotype (designated as M1) is a promising therapeutic option for different types of cancer [7, 8]. TAM re-polarization has been achieved by toxic triggers such as chemo- and radiotherapy, and it is suggested that such perturbations lead to further modulation of the tumor microenvironment, resulting in immune activation.

Abbreviations: TAM, Tumor-associated macrophage; PDT, Photodynamic therapy; ROS, reactive oxygen species; AlPc, aluminum phthalocyanine; qRT-PCR, quantitative reverse transcription polymerase chain reaction; LPS, lipopolysaccharide; DAPI, 4′,6-diamidino-2-phenylindole; PMA, Phorbol-12-myristat-13-acetate; SEM, standard error of the mean; MFI, mean fluorescence intensity; EPR, enhanced permeability and retention

* Corresponding author.
E-mail address: rainer.wittig@ilm-ulm.de (R. Wittig).
[1] Present address: University Hospital, Research Center, Am Klinikum 1, 07747 Jena, Germany

https://doi.org/10.1016/j.jphotobiol.2018.06.015
Received 19 April 2018; Received in revised form 6 June 2018; Accepted 23 June 2018
Available online 25 June 2018
1011-1344/

Photodynamic therapy (PDT) is a promising method for the localized treatment of tumors which are accessible by light. Here, photosensitizing molecules (photosensitizers, PS) preferentially accumulate in cancer tissue, followed by targeted illumination, which minimizes adverse effects when compared to alternative systemic treatments. During PDT, energy transfer from PS to molecular oxygen leads to the formation of reactive oxygen species (ROS), which instantly react with molecules in the near vicinity, resulting in cell damage. The majority of tumoricidal effects are ascribed to the highly reactive singlet oxygen, which is a dominant ROS during photodynamic therapy [9]. PDT functions through both cancer-cell directed phototoxicity and modulation of the immune system [10]. Besides the tumor cells themselves, TAM are also targets of PDT, because their inherent phagocytic activity leads to the accumulation of PS previously administered to a patient. It has been suggested that the immunostimulating effects of PDT are at least partially mediated by modulation of M2-polarized macrophages. In response to PDT these are either killed or activated [11], resulting in a change in the phenotypic balance of TAM towards the pro-inflammatory and immunogenic M1-polarization state.

Recently, we investigated the photodynamic activity of crystalline, nonfluorescent nanoparticles made from aluminum phthalocyanine (AlPc) both in vitro [12] and in vivo [13]. Here, AlPc-derived fluorescence was predominantly detectable in macrophages and rejected skin autografts, leading to the hypothesis that such nanoparticles may preferentially fluoresce in injured tissue. The clinically approved PS meta-tetrahydroxyphenylchlorin (mTHPC, Temoporfin) reveals a planar molecular structure similar to AlPc and was therefore suspected to reveal similar dissolution/fluorescence characteristics when administered in crystalline, nonfluorescent formulation. In a recent in vitro study using such Temoporfin-derived nanoparticles we demonstrated a preferential uptake into macrophages when compared to fibroblasts [14]. In the present study, we aimed at the characterization of photodynamic effects of these nanoparticles in macrophage populations featuring different polarization states.

2. Materials & Methods

2.1. Photosensitizers

Temoporfin raw material for the preparation of crystalline nanoparticles was provided by biolitec research GmbH (Jena, Germany). The nanoparticles N2 and N3 were prepared as described previously [14]. Briefly, Temoporfin from DMSO- (N2) and ethanol-solution (N3) was precipitated by dissolution in excess water and subsequently dispersed by ultrasound exposition. Particle sizes were 400 ± 43.08 nm for N2 and 200 ± 17.93 nm for N3, respectively. The zeta-potentials were slightly negative, with −18.4 mV for N2 and − 21.6 mV for N3. Foslip is a liposomal Temoporfin formulation containing a 9:1 mixture of dipalmitoylphosphatidylcholine (DPPC) and dipalmitoylphosphatidylglycerol (DPPG; > 99% purity). The freeze-dried product was provided by biolitec research GmbH and reconstituted with water as recommended by the manufacturer. The resulting suspension contained 20 mg of phospholipids, 1.5 mg Temoporfin, and 50 mg glucose per ml. The average hydrodynamic diameter of the liposomes was approx. 130 nm.

2.2. Cell Culture and Macrophage Differentiation/Polarization

The THP-1 monocyte cell line (Cell Lines Service, Eppelheim, Germany) was originally isolated from peripheral blood of a 1 year old boy suffering from acute monocytic leukemia [15]. Cells were grown in RPMI 1640 medium (Life Technologies, Darmstadt, Germany) supplemented with 10% fetal bovine serum (Applichem, Darmstadt, Germany), 1% streptomycin/penicillin (Life Technologies), 2 mM glutamine (Life Technologies) and 1 mM sodium pyruvate (Life Technologies) under standard conditions (37 °C, 5% CO_2) in suspension at densities from 10^5 to 8×10^5 cells/ml. Differentiation of THP-1 monocytes to adherently growing macrophages (M0) was achieved by seeding of 10^5 cells/ml in presence of 100 nM Phorbol-12-myristat-13-acetate (PMA) during 72 h. Forty-eight hours post PMA-incubation, media were additionally supplemented with human recombinant Interferon gamma (IFN-γ, 20 ng/ml, Thermo Fisher Scientific, Karlsruhe, Germany) and lipopolysaccharide (LPS, 100 ng/ml, Sigma-Aldrich, Steinheim, Germany) for M1-polarization, or with human recombinant Interleukin-4 (IL-4, 20 ng/ml, R&D Systems, Wiesbaden, Germany) and human recombinant Interleukin-13 (IL-13, 20 ng/ml, R&D Systems) for M2-polarization, respectively. Cells were then Temoporfin-treated and analysed as described below. For quantitative reverse transcription polymerase chain reaction (qRT-PCR)-mediated evaluation of polarization marker expression, cells were cultured for additional 24 h in fresh medium without supplements.

2.3. Cytotoxicity Studies

For cytotoxicity studies, THP-1 cells were seeded at 267 cells/mm^2 in 96-well microplates. Whereas the monocyte population was grown for three days without treatment, macrophage differentiation and polarization was conducted during three days as described in 2.2. Three days post seeding, cell populations were incubated with Temoporfin formulations (Foslip, N2, N3) in the absence of polarization triggers for further 24 h in the dark. Cell viability was determined using the Cell Titer Blue assay (Promega, Mannheim, Germany) as recommended by the manufacturer. Resorufin-mediated fluorescence (excitation: 544 nm, detection: 590 nm), the intensity of which correlates to the number of viable cells per well, was quantified using a Fluostar Omega microplate reader (BMG Labtech, Ortenberg, Germany). Fluorescence intensity values were blank corrected and viability was calculated as a percentage of the untreated control. For each sample, three independent experiments were performed in quadruplicates.

2.4. Photodynamic Treatment

For phototoxicity studies, THP-1 monocytes were seeded at 267 cells/mm^2 in 4-well plates. Macrophage differentiation and polarization was conducted as described in 2.2. Temoporfin formulations were added at 0.5 μM for 24 h as described in 2.3. Temoporfin containing media were replaced by fresh media, and cells were subjected to illumination using a Ceralas PDT Laser (λ = 652 nm, 25 mW/cm^2, 5 J/cm^2, biolitec research GmbH). Twenty-four hours post illumination, cells were assayed for viability as described in 2.3. At least three independent experiments were performed in quadruplicates. For the analysis of a photodynamic modulation of macrophage polarization states, cells were seeded and differentiated/polarized as described in 2.2. Subsequently, cells were incubated with nanoparticle N2 (0.1 μM) for 24 h in the dark. Temoporfin-containing media were replaced by fresh media and cells were exposed to laser light (3 J/cm^2) as described above. Two hours post photodynamic treatment, cells were harvested and subjected to RNA-preparation for quantitative reverse transcription polymerase chain reaction (qRT-PCR) as described in 2.7.

2.5. Flow Cytometry

THP-1 cells were seeded and differentiated/polarized as described in 2.2. Three days post seeding, the cells were incubated with Temoporfin formulations (0.5 μM) for 2 or 24 h. At the end of the incubation period, cells were harvested, washed and resuspended in flow cytometry buffer (PBS, 1% BSA). For detection of dead cells, 4′,6-diamidino-2-phenylindole (DAPI) was added at 0.5 μg/ml. Flow cytometry was conducted using a CyFlow Space instrument (Sysmex-Partec, Görlitz, Germany) and FloMax 3.0 software (Sysmex-Partec). Excitation of DAPI- and Temoporfin-mediated fluorescence was conducted using a 405 nm Laser, detection was carried out using a BP455 filter for DAPI